

## POSTER PRESENTATION

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# Differential phosphorylation of LZ+/LZ- MYPT1 isoforms by PKGI $\alpha$ : implication for vascular reactivity

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*From* 5th International Conference on cGMP: Generators, Effectors and Therapeutic Implications  
Halle, Germany. 24-26 June 2011

## Background

MLC phosphatase is a trimeric enzyme composed of a catalytic subunit, a 20-kDa subunit of unknown function, and a myosin targeting subunit (MYPT1). During NO stimulation, PKGI $\alpha$  mediated phosphorylation of MYPT1 increases MLC phosphatase activity, which produces a decrease in force. Further, alternative splicing of a 3' exon produces two MYPT1 isoforms, which differ by the presence or absence of a leucine zipper (LZ); a LZ+ MYPT1 isoform is required for PKGI $\alpha$  induced smooth muscle relaxation.

## Results

To examine the influence of MYPT1 structure on the ability of PKGI $\alpha$  to phosphorylate the protein, we used two MYPT1 fragments, which differed only by the presence (MYPT1LZ+) or absence (MYPT1LZ-) of the LZ. Purified PKGI $\alpha$  phosphorylated MYPT1LZ+, but not MYPT1LZ-. Following phosphorylation, MYPT1LZ+ predominantly existed as a di-phosphorylated protein, and mass spectrometry identified S<sup>668</sup> and S<sup>695</sup> as PKGI $\alpha$ -mediated phosphorylation sites. To examine the relative rates of S<sup>668</sup> vs S<sup>695</sup> MYPT1 phosphorylation, these residues were mutated to either A or D. The rates of D<sup>668</sup> and A<sup>668</sup> MYPT1 phosphorylation were similar and slow. The D<sup>695</sup> MYPT1 mutant had the highest rate of phosphorylation, while the rate of phosphorylation of the A<sup>695</sup> MYPT1 mutant was intermediate between that for the D<sup>695</sup> MYPT1 and either A<sup>668</sup> or D<sup>668</sup> MYPT1.

## Conclusion

These results suggest that PKGI $\alpha$ -mediated phosphorylation of S<sup>695</sup> is slower than S<sup>668</sup>, and could suggest that PKGI $\alpha$ -mediated phosphorylation of S<sup>668</sup> is physiologically significant for the regulation of MLC phosphatase activity. Further, MYPT1 structure has an important role in the regulation of vascular tone, and differential tissue expression of LZ+/LZ- MYPT1 isoforms contributes to the diversity in the sensitivity of smooth muscle to NO mediated vasodilatation.

Published: 1 August 2011

doi:10.1186/1471-2210-11-S1-P78

**Cite this article as:** Yuen et al.: Differential phosphorylation of LZ+/LZ- MYPT1 isoforms by PKGI $\alpha$ : implication for vascular reactivity. *BMC Pharmacology* 2011 **11**(Suppl 1):P78.

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